Highly sensitive immunoassay with dual signal amplification systems of redox cycling in nanospace and cascade reaction

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Sensitivity improvement for immunoassay is desired to detect early stage of infection. In this study, according to our previous report[1], we improved the sensitivity for immunoassay using dual signal amplification systems of redox cycling in nanospace[2] and *Limulus* amebocyte lysate (LAL) reaction[3] which is one of cascade reaction induced by endotoxin. Immunoassay for goat IgG as a model analyte was performed with endotoxin-labeled antibody. After immunoassay, LAL reaction was performed, and *p*-aminophenol (pAP) liberated from peptide-conjugated *p*-aminophenol (LGR-pAP) at the last step of LAL reaction was detected using redox cycling in nanospace (Fig. 1).

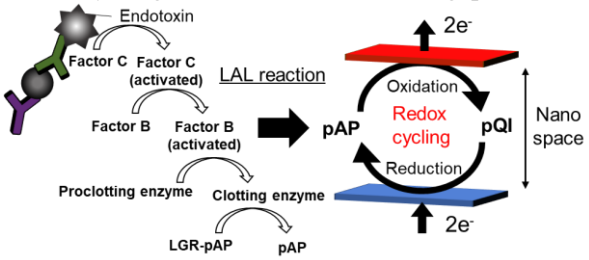

Fig. 1 Schematic illustration of this research

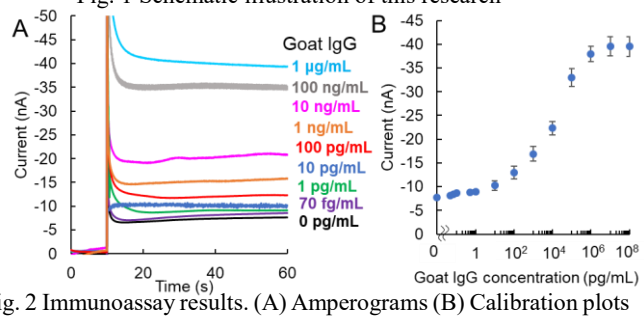

Fig. 2 Immunoassay results. (A) Amperograms (B) Calibration plots

A nanogap device with a pair of ring electrodes facing each other across a 190 nm gap was fabricated with photolithography and sputtering according to our previous report[1]. Immunoassay for goat IgG was performed on carboxylated magnetic beads (d=1.0 μm). First, beads were conjugated with 60 μg/mL anti-goat IgG antibody using carbodiimide cross-linker. Secondly, beads were incubated with 2 mg/mL bovine serum albumin to block non-specific reaction. Then, beads were reacted with goat IgG. After that, beads were reacted with endotoxin-labeled anti-goat IgG antibody. Finally, LAL reaction with 0.5 mM LGR-pAP was performed for 1 h at 37°C. The pAP liberated from LGR-pAP was detected using the nanogap device with the potential of the bottom electrode biased at -0.2 V, while the top electrode stepped from -0.2 to 0.5 V.

Fig. 2 shows amperograms obtained from the bottom electrode (A) and calibration plot obtained by subtracting the current at 9.96 s from the current at 60 s (B). The reduction current increased with the increase of goat IgG concentration. From the result of IgG assay, the limit of detection was calculated to be 70 fg/mL (467 aM) as concentration corresponding to three times the standard deviation of 0 pg/mL. Our strategy can provide highly sensitive immunoassay platform for clinical diagnosis.

1) K. Ito et al., Analyst, 2019, 144, 3659-3667. 2) M. A. G. Zevenbergen et al., Nano Lett., 2007, 11, 2881-2886.
3) K. Y. Inoue et al., Innate Immun., 2012, 18. 343-349.